Subacute sclerosing panencephalitis and multiple sclerosis: in vitro measles immunity and sensitization to myelin basic protein

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Three children with subacute sclerosing panencephalitis (SSPE), 12 patients with multiple sclerosis (MS) and 12 healthy persons were studied by the macrophage migration inhibition factor (MIF) assay with measles and rubella antigens and with myelin basic protein. For the SSPE patients the mean migration indexes ± standard deviation were 44.1 ± 10.9 for measles antigen, 38.7 ± 12.3 for rubella antigen and 49.8 ± 25.7 for myelin basic protein; for the MS patients the indexes were 103.0 ± 10.6, 93.8 ± 15.0 and 89.3 ± 19.9; and for the healthy subjects the indexes were 68.8 ± 22.6, 77.7 ± 31.3 and 100.4 ± 6.5.

The results of this study showed increased cellular immunity to measles and rubella in SSPE patients as compared with healthy persons, and absence of immunity to measles in MS patients. Patients with MS showed hypersensitivity to myelin basic protein during clinical exacerbations that was not associated with changes in immunity to measles, whereas all SSPE patients showed a significant response regardless of stage of illness.

On a étudié chez 3 enfants souffrant de leucoencéphalite sclérosante subaiguë (LESS), chez 12 patients atteints de sclérose en plaques (SP) et chez 2 personnes saines la réponse aux antigènes de la rougeole et de la rubéole, et à la protéine myélinique, par l'épreuve du facteur inhibant la migration des macrophages (FIM). Pour les patients porteurs de LESS, les indices moyens de migration ont été de 44.1 ± 10.9 pour l'antigène de la rougeole, 38.7 ± 12.3 pour l'antigène de la rubéole et de 49.8 ± 25.7 pour la protéine myélinique; pour les patients atteints de SP, les indices ont été de 103.0 ± 10.6; 93.8 ± 15.0 et 89.3 ± 19.9; chez les sujets sains, les indices ont été de 68.8 ± 22.6, 77.7 ± 31.3 et 100.4 ± 6.5.

Les résultants de cette étude ont révélé une augmentation de l'immunité cellulaire à la rougeole et à la rubéole chez les patients atteints de LESS, par rapport aux personnes saines, et une absence d'immunité à la rougeole chez les porteurs de SP. Les patients atteints de SP ont manifesté une hypersensibilité à la protéine myélinique pendant les périodes d'exacerbation clinique qui n'a pas été associé à un changement de l'immunité à la rougeole, alors que tous les patients atteints de LESS montraient une réponse significative, quel que soit le stade de la maladie.

Both subacute sclerosing panencephalitis (SSPE) and multiple sclerosis (MS) have been hypothesized as being related to measles virus infection.[1-16] Measles-like inclusion bodies, first seen in brain tissue of a patient with SSPE by Dawson,[1] have been shown to contain paramyxovirus-like tubules.[2,3] Serum of patients with SSPE has been found to contain an increased titre of measles antibodies,[4,5] and the virus has been isolated from affected brain tissue.[6-8] Consequently, impaired immunity to measles has been suggested as a predisposing factor to SSPE.[9-11] While confirmation of Adams and Brown's[12] report of inclusion bodies in brain tissue in MS is lacking,[13-16] measles antibody titres have been found to be increased in MS.[17-20] As with SSPE[9] the question of impaired cellular immunity to measles virus as a contributing factor in MS has been raised.[21-24] Because of varying results for both disorders in previous studies we sought to investigate the status of measles and rubella immunity in these diseases by means of an established assay technique — the macrophage migration inhibition factor (MIF) assay.

Methods

Patients

With SSPE: Three patients with SSPE, a girl aged 8 years with stage 3 (Ford's staging) disease and two boys aged 11 years, one with stage 1 and the other with stage 3 disease, with classical clinical and electroencephalographic findings, were studied. Four siblings of patient 2, including a fraternal twin, his parents and two unrelated children were studied concomitantly. Each patient had a history of clinical measles, two in the first 2 years of life and the third at age 5 years. Thymic tissue was implanted in two patients and MIF assays for cellular immunity to measles and rubella and for sensitization to myelin basic protein were done before specific immunotherapy was initiated; two assays were done in patient 1, four in patient 2 and four in patient 3.

With MS: Twelve patients aged 16 to 33 years matched with healthy subjects for sex, age and ethnic background were studied. Three were studied within 4 weeks of an acute exacerbation of illness and nine were studied 3 to 9 months after an attack.

Healthy: Twelve persons aged 16 to 30 years were studied concomitantly with the SSPE and MS patients; at least one healthy individual was studied concomitantly with each patient.

Tests

The titres of complement-fixing (CF) antibody to measles virus and of hemagglutination-inhibiting (HI) antibodies to rubella virus were determined in the serum or cerebrospinal fluid (CSF), or both, in all subjects.

The MIF assay system described by Rocklin, Myers and David[25] was used with minor modifications designed to reduce cell handling. Heparinized peripheral venous blood from the subjects was sedimented with 5% dextran for 30 to 60 minutes at 37°C while in upright 60-mL plastic disposable syringes. Buffy coats were expressed by connecting tubing directly onto cotton in glass test tubes and incubated for 30 minutes at 37°C. The lymphocytes were then washed from the cotton with twice the volume of tissue culture fluid (TC199; Microbiological Associates, Bethesda, Maryland). Fluid containing the lymphocytes was aspirated into a second plastic syringe by a second connecting plastic tube. The lymphocytes of 95% purity so obtained were subsequently washed three times with TC199 without nutrient serum and were cultured in aliquots of 3×10^6/mL with and without antigen for 3 days. Culture supernatants were harvested daily, dialyzed for 72 hours and assayed for the presence of MIF with the use of normal guinea pig peritoneal macrophages packed into capillary tubes. Controls for antigen toxicity were included in every experiment. Areas of macrophage migration were measured by projection and planimetry, and percent migration

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was calculated by the formula

$$\% \text{ migration} = X/Y \times 100$$

where X = area of migration in supernatant with antigen ÷ area of migraton in medium with antigen, and Y = area of migration in supernatant without antigen ÷ area of migration in medium without antigen. The formula nullifies the effects of nonspecific cytotoxic factors in culture supernatants and inhibition or stimulation induced by the antigens.

Lymphoblast transformation

In separate studies lymphocytes from patients with SSPE and healthy controls were stimulated with phytohemagglutinin (PHA; Burroughs-Wellcome).

Antigens

Measles (Enders–Edmonston 84F strain) and rubella (Gilchrist 3HK-21-cc/13 strain) antigens (supplied by Flow Laboratories, Rockville, Maryland) were used in a 1/50 concentration in TC199 without nutrient serum.

Human myelin basic protein with a high degree of purity was prepared in a concentration of 10 μg/mL.

Results

Antibodies to measles virus

At the onset of SSPE the titres of CF antibody to measles virus in the three patients were between 1/256 and 1/8192 in the serum and between 1/8 and 1/128 in the CSF. During the course of the illness the titres increased greatly, to between 1/160 and 1/8192. Implantation of thymic tissue did not appear to affect the in vitro findings in the two recipients with SSPE, nor was there any obvious clinical response.

Of the patients with MS two had no detectable CF antibody to measles, seven had a titre of 1/16, two had a titre of 1/32 and one had a titre of 1/64.

Six of the healthy persons had no detectable CF antibody to measles, four had a titre of 1/8 and two had a titre of 1/16.

Antibodies to rubella virus

Titres of HI antibody to rubella virus did not differ between the three groups.

Migration indexes

The migration indexes for measles and rubella antigens and for myelin basic protein are shown in Figs. 1 to 3. Mean values ± standard deviation were 44.1 ± 10.9, 38.7 ± 12.3 and 49.8 ± 25.7 respectively for the SSPE patients, 103.0 ± 10.6, 93.8 ± 15.0 and 89.3 ± 19.9 for the MS patients and 68.8 ± 22.6, 77.7 ± 31.3 and 100.4 ± 6.5 for the healthy subjects. Results with higher dilutions of measles antigens were not substantially different. Student's *t*-test for unpaired samples showed that the responses to measles antigen in the SSPE patients were significantly different from those in the MS patients ($P < 0.001$) and the healthy subjects ($P < 0.005$), and the responses in the MS patients were significantly different from those in the healthy subjects ($P < 0.005$). The responses to rubella antigen in the SSPE patients also differed significantly from the responses in the MS patients and the healthy subjects ($P < 0.001$); however, the responses in the MS patients and the healthy subjects did not differ significantly. In the SSPE patients and the healthy subjects there was no significant difference in the responses to measles and rubella antigens. SSPE patients showed a significantly greater response to myelin basic protein than did MS patients or healthy subjects ($P < 0.001$).

The results for one patient with SSPE, family members and control subjects studied serially are shown in Table I. A fraternal twin demonstrated significant MIF production in response to measles antigen and myelin basic protein at the time of onset of his brother's illness, but the former was not clinically ill. Repeated assays subsequently showed this hyperimmune state to be transient. All of the siblings, but not the parents, showed more MIF production at the time of onset of the patient's illness. These findings suggest that the agent that caused the illness may have affected the patient's siblings concomitantly, resulting in an immune response but no obvious clinical illness. All the siblings had had measles at the same time 4 years previously.

These results indicate that, in contrast to an increased MIF production in SSPE, the lymphocytes of patients with clinical MS do not produce substantial amounts of this lymphokine and therefore the patients appear to lack normal cellular immunity to measles. In one unrelated healthy person tested with measles antigen the migration index was 29 which is similar to those of the patients with SSPE. Immunity to rubella did not differ between the MS patients and the healthy persons but appeared to be increased in the SSPE patients. All patients showed pronounced sensitization to myelin basic protein. In three patients with acute exacerbations of MS the assay with measles antigen produced results (migration indexes of 79, 66 and 50) that did not differ from those in the remainder of the group.

Relation of antibody titres and migration indexes

The relation of antibody titres and migration indexes in MS patients and controls is shown in Table II.

Biopsy findings

Brain: Thin-section electron microscopy of a parietal lobe biopsy specimen showed nuclear inclusion bodies in several neurons to be composed of tubular structures typical of paramyxovirus.

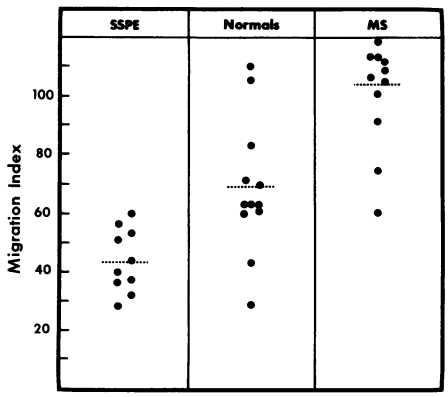

FIG. 1—Cellular immunity to measles virus (Enders–Edmonston 84F strain) in patients with subacute sclerosing panencephalitis (SSPE) or multiple sclerosis (MS) and in healthy subjects ("normals"). Mean values indicated by interrupted lines.

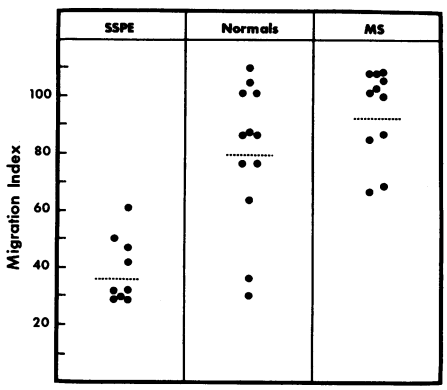

FIG. 2—Cellular immunity to rubella virus (Gilchrist 3HK-21-cc/13 strain).

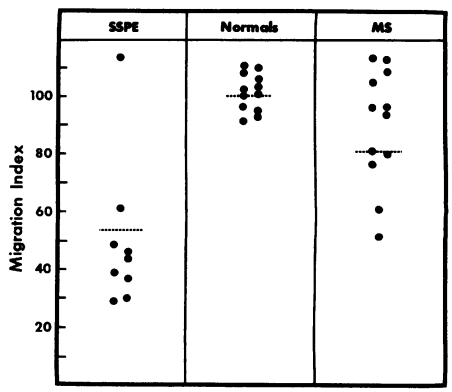

FIG. 3—Cellular sensitization to human myelin basic protein.

Table I—Cellular sensitization to measles antigen (MA) and myelin basic protein (MBP), as assessed from migration indexes, in patient with subacute sclerosing panencephalitis (SSPE), family members and unrelated healthy control subjects

Months after onset of SSPE	Migration index															
	Patient		Twin		Sibling 2		Sibling 3		Mother		Father		Control 1*		Control 2*	
	MA	MBP	MA	MBP	MA	MBP	MA	MBP	MA	MBP	MA	MBP	MA	MBP	MA	MBP
1	36	38	43	41	26	114	–	–	117	96	–	–	70	100	44	98
2	30	67	–	–	117	–	–	–	–	–	–	–	61	96	40	92
3	119	–	–	–	111	–	–	–	–	–	–	–	87	–	53	102
4	26	–	37	–	–	–	77	–	90	100	64	110	–	–	–	–
5	40	–	42	–	–	–	81	–	–	–	–	–	–	–	–	–
6	40	57	57	96	–	–	85	–	–	–	–	–	–	–	–	–
12	56	60	67	104	72	100	80	–	–	–	–	–	60	90	65	96

* The children studied at 3 and at 12 months were different from those studied on the other occasions.

Table II—Humoral and cellular immunity to measles in multiple sclerosis

Titres of complement-fixing antibody to measles virus	Migration index	
	Patients with multiple sclerosis	Healthy controls
0	60, 75	70, 72, 63, 44, 84, 63
1/8	–	110, 105, 29, 60
1/16	92, 101, 50, 109, 122, 112, 113	63, 61
1/32	113, 106	–
1/64	94	–

Lymph node: The lymph node architecture in patients 2 and 3 with SSPE was completely normal.

PHA stimulation in SSPE

Lymphocytes from patients and controls showed no significant difference in incorporation of tritiated thymidine.

Discussion

We have demonstrated anergy to the Enders–Edmonston strain of measles virus in MS but increased immunity in SSPE. With the same antigen we found very high titres of CF antibody during the course of SSPE but only slight elevation of titres in MS. Immunity to rubella virus did not differ significantly between MS patients and healthy controls, but there was an unexpected, substantial increase in cellular but not humoral immunity in SSPE patients. In SSPE hyperimmunity to measles appears to be related to hypersensitization to myelin basic protein, whereas in MS lack of measles immunity shows no correlation with sensitization to myelin basic protein or phase of illness.

Elevated titres of antibody to measles in serum and CSF in SSPE were demonstrated first by Connolly, Haire and Madden[4] in 1971 and since then have been detected in virtually all studies. Bouteille and colleagues,[2] as well as others,[3] have found paramyxovirus-like particles in brain tissue from patients with SSPE. The agent was subsequently recovered by cell fusion techniques.[6-11]

Saunders and associates,[26] using an undefined measles antigen, found a marked increase in incorporation of ^{3}H-thymidine in lymphocytes of an 8-year-old patient with SSPE, as compared with a control subject. Moulias, Reinert and Goust,[27] however, were unable to confirm these observations and reported a lack of lymphoblastic transformation in response to measles antigen in SSPE; this apparently was reversed after normal lymphocytes were infused into the patient. Other workers using the same type of assay system have also failed to observe a response to measles antigen in SSPE.[28,29] The lymphoblastic transformation assay system and measles antigen have, in other hands, also failed to give positive results.[9] However, if measles or SSPE virus is "fixed" to cells this assay yields positive results.[9,30]

The MIF assay first was used to demonstrate immunity to measles by Mizutani and colleagues[31] in 1971. They found that measles immunity was greater in an SSPE patient than in two healthy controls, findings in keeping with the earlier observations by Saunders and associates.[26] The in vitro findings correlated with the results of skin tests carried out simultaneously. Ahmed and colleagues,[30] using the same indirect MIF assay system[25] as we did and a modified direct assay system, have obtained positive results in three cases of SSPE with SSPE virus and in two of four cases with autoclaved measles virus. The results of Ahmed and Mizutani and their colleagues and our results, all with the same MIF assay system, have demonstrated consistently a normal or increased response to measles antigen.

Although there is no clinical evidence to suggest that infection with measles virus may lead to MS, there is epidemiologic evidence[32] as well as evidence dependent on pathologic and immunologic findings to support the belief that measles is important in the pathogenesis of this disease. In 1962 Adams and Imagawa[17] first showed an increased frequency of elevated titres of antimeasles antibodies in persons with MS. Since then almost every study of this subject has supported their finding.[18,19] In 1974 Arnason[20] showed that elevated titres of antibody to measles virus were related to the presence of the HLA-A3 histocompatibility antigen, which occurs with increased frequency in patients with MS. Despite the report by Adams and Brown[12] in 1969 that intranuclear inclusion bodies resembling measles inclusion bodies were present in neurons and glial cells, these findings have not been supported by other investigations.[13-16] Prineas[13] and others[14,16] have observed "tubules" in the cytoplasm of mononuclear cells near acute lesions, but these structures did not appear to be paramyxoviruses and were observed in several other conditions. The immune electron microscopic studies of Dubois-Dalcq, Schumacher and Sever,[14] particularly, argue against these structures, when seen in brain tissue of patients with MS, as having any homology with measles virus.

Utermohlen and Zabriskie[22] and Jersild and associates[23] have reported evidence of impaired cellular immune responses to measles, as measured by direct cell migration assays. In contrast to these observations, an earlier study by Knowles and Saunders[21] in 1970 with lymphoblastic transformation as-

says showed apparently normal responses in both MS patients and healthy subjects. While Offner and colleagues,[24] using a whole-blood culture lymphoblastic transformation method, found responses in neither MS patients nor healthy controls, another such study demonstrated positive responses.[33]

Results of assays in our MS patients were distinctly different from those in the healthy subjects, which in turn were different from those in the SSPE patients. The observed responses to measles antigen in MS did not parallel the responses to myelin basic protein, despite the reported similarity of the two antigens.[34] Results of assays with both antigens bore no relation to the titres of CF antimeasles antibody. The difference in cellular immunity to measles seen in the SSPE patients, the MS patients and the healthy subjects constitutes additional evidence for the specificity of the response to the antigen.

Sensitization to myelin basic protein was seen in all three SSPE patients, in a sibling of one patient (no. 2) with SSPE and in two of the three patients studied within 4 weeks of an acute exacerbation of MS. Although the siblings of patient 2 exhibited a greater response to measles antigen as compared with unrelated healthy control subjects, they did not exhibit sensitization to myelin basic protein. However, with the passage of time the immune response to measles seemed to be less striking in the patient and his siblings. These results in MS are similar to those of other studies.[35,36] The presence of demyelinating lesions in the brain of patients with SSPE and MS makes this finding not unexpected. In our previous study[36] in vitro evidence of acquired sensitization in patients who had had a stroke or head injury was not as pronounced as in patients with acute exacerbations of MS. The findings in the SSPE patients are of the same order as those in the patients with acute exacerbations of MS. Perhaps the demyelinization in SSPE, as in canine distemper, is a consequence of pathogenetic immune mechanisms initiated by the presence of virus and not necessarily a direct effect of the virus. However, sensitization to myelin basic protein in SSPE, in contrast to that in MS, appears not to be related to the phase of the illness.

Progressive rubella encephalitis, an illness resembling SSPE, has been recognized as complicating congenital and perhaps acquired rubella infections.[37,38] The surprising finding of a substantial increase in cellular immunity to rubella in all three patients with SSPE, and in the twin of one of the patients, but not of rubella antibodies, raises a question as to the identity of the viruses responsible for illness in classical SSPE and in progressive rubella encephalitis. The antigen responsible for the results in SPPE may be a virus protein or a virus-induced cell protein. The assay results in the three groups in our study suggest that the antigen is more likely to be a viral antigen and that the SSPE virus may be antigenically unique. However, the findings do not rule out a genetically determined or acquired host-dependent response factor.

The demonstration by Koprowski, Barbanti-Brodano and Katz[39] of papovavirus-like particles in cultures of brain tissue from SSPE patients raises the question whether such an agent may function as a helper virus. Increased titres of antibody to Epstein–Barr virus in 8 of 12 patients with SSPE studied consecutively by Joncas and colleagues,[40] in 3 other patients who had had infectious mononucleosis[41] and in another isolated case[42] further complicate the matter. These observations suggest that a variety of agents may cause reactivation of a latent paramyxovirus in the central nervous system. This may be true not only for SSPE but also for progressive rubella encephalitis. The findings of the MS-associated agent (MSAA) by Carp and associates,[43] apparently confirmed by Koldovsky and colleagues,[44] suggests that a similar situation might pertain in MS.

Our observations indicate increased immunity to measles and rubella virus in SSPE patients, correlated with hypersensitization to myelin basic protein. They suggest that a hyperimmune response to measles and rubella virus may precipitate hypersensitization to myelin basic protein, which in turn may be a factor in tissue destruction. Absence of cellular immunity to measles does not appear to be a factor in SSPE; however, anergy to measles virus in MS may contribute to the pathogenesis of this disease.

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Influence of age and previous use on diazepam dosage required for endoscopy

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In 19 patients (10 men and 9 women) a 22-fold variation was observed in the intravenous dose of diazepam necessary as preparation for endoscopy (median dose, 20 mg; range, 5 to 110 mg). Analysis of plasma samples for diazepam and N-desmethyldiazepam revealed that the clinical response did not relate to the rate or character of initial drug distribution. There was a high correlation ($r = 0.96$) between the dose and the plasma concentration 10 minutes after administration. Users of diazepam displayed tolerance to its pharmacologic effects, requiring a significantly larger ($P < 0.05$) dose than nonusers (median doses, 35.0 mg and 14.5 mg respectively). Older patients required less than younger patients ($r = -0.54$, $P < 0.05$). The variation between individuals in the dose of diazepam required as preparation for endoscopy cannot be explained by variation in drug disposition but instead reflects previous diazepam use, age and probably differences in sensitivity at the site or sites of drug action.

Chez 19 patients (10 hommes et 9 femmes), on a constaté une variation de 22 fois dans la dose intraveineuse de diazépam nécessaire à la préparation à l'endoscopie (dose médiane, 20 mg; variation de 5 à 110 mg). L'analyse du diazépam et du N-desméthyldiazépam dans les échantillons plasmatiques a révélé que la réponse clinique n'était pas reliée au taux ou au caractère de la distribution initiale du médicament.

On a observé une forte corrélation ($r = 0.96$) entre la dose administrée et la concentration plasmatique atteinte 10 minutes après l'administration. Les utilisateurs de diazépam ont démontré une tolérance à ses effets pharmacologiques, alors qu'ils ont nécessité une dose significativement plus grande ($P < 0.05$) que ceux qui n'en prenaient pas (doses médianes, 35.0 mg et 14.5 mg, respectivement). Les patients plus âgés en ont nécessité moins que les patients plus jeunes ($r = -0.54$, $P < 0.05$). Les variations individuelles des doses de diazépam nécessaires à la préparation à l'endoscopie ne peuvent être expliquées par une variation dans l'élimination du médicament, mais elles reflètent plutôt l'emploi préalable de diazépam, l'âge, et probablement des différences de sensibilité au lieu d'action du médicament.

The use of intravenous diazepam for sedation during peroral gastrointestinal endoscopy is well established but there is a wide variation in the dose required for this procedure. We undertook a study to determine the extent of the dose range and to assess some of the factors that may influence the immediate response to the drug.

There is evidence that age[1] and sex[2] may affect a person's sensitivity to the pharmacologic action of drugs, and acute[3] and chronic[4] tolerance to both the psychomotor and sedative effects of psychoactive drugs have been described. It is of interest, therefore, to determine how these factors determine drug action in a clinical setting. Endoscopy provides one of the few situations where a distinct, consistent, clinical endpoint is achieved with diazepam, namely the point at which relaxation is sufficient to permit the procedure.

Methods

The study group consisted of both in- and outpatients who underwent gastrointestinal endoscopic examination at Toronto Western Hospital. The investigation was approved by the human ethics committee of the hospital and the participants granted informed consent. Excluded from the study were patients requiring emergency endoscopy and those with a hemoglobin value of less than 12 g/dL. The alleged use of diazepam during the preceding year was recorded for each patient.

Patients were classified as users of diazepam if they alleged taking more than 2 mg/d for more than 7 days during the previous year and a blood sample was found to be positive for diazepam or N-desmethyldiazepam, or both (Table I). (The terminal half-life of diazepam in normal subjects is 1 to 2 days, whereas for N-desmethyldiazepam it is approximately twice as long.[5]) If neither of these conditions were met, patients were classified as nonusers. A few patients satisfied only one of the two criteria for users and were not included in the study.

The surface area of the patients was determined from a nomogram after height and weight had been measured.

The patients were selected and prepared for endoscopy on the basis of the accepted clinical indications. Premedication consisted of intramuscular doses of meperidine, 75.0 mg, and atropine, 0.6 mg, given 30 minutes prior to the procedure and xylocaine was applied by spray to the pharynx. A butterfly needle was introduced into a forearm vein for blood sampling. The plasma samples thus obtained were frozen until analysed on an electron-capture gas chromatograph (HP-7620A) with an HP-3380 integrator (Hewlett-

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